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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 35/78, 31/35

A1

(11) International Publication Number: **WO 99/29331**

(43) International Publication Date: 17 June 1999 (17.06.99)

(21) International Application Number: PCT/EP98/07662

(22) International Filing Date: 30 November 1998 (30.11.98)

(30) Priority Data:
MI97A002690 4 December 1997 (04.12.97) IT

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THE USE OF PHOSPHOLIPID COMPLEXES OF EXTRACTS OF *VITIS VINIFERA* AS ANTI-ATHEROSCLEROTIC AGENTS

(57) Abstract

The phospholipid complexes of extracts of *Vitis vinifera* are useful for the prevention and the treatment of atherosclerotic pathological conditions.

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THE USE OF PHOSPHOLIPID COMPLEXES OF EXTRACTS OF VITIS VINIFERA AS ANTI-ATHEROSCLEROTIC AGENTS

The present invention relates to the use of phospholipid complexes of oligomeric proanthocyanidins containing from 2 to 7 catechin units extracted from *Vitis vinifera* for the preparation of medicaments for the treatment and the prophylaxis of atherosclerosis, and of miocardiac and cerebral infarctions.

The extracts of *Vitis vinifera* are already known and used in the therapy of cardiovascular disorders connected with venous insufficiency, in the treatment of impaired conditions of capillary permeability and resistance and in cicatrization. These extracts, which can be obtained from the seeds of the plant as described in GB-A-1,541,469 or in FR-A-2,092,743, are a mixture of polyphenols such as epicatechin and its polymerization products, in part esterified at the C-3 hydroxyl of the monomer with gallic acid. The phospholipid complexes of the extracts of *Vitis vinifera* are described in US-4,963,527 and are at present commercially available under the trade mark Leucoselect.

It has now surprisingly been found that the complexes prepared according to US-4,963,527 exert, when administered systemically, preferably by the oral route, a marked anti-atherosclerotic activity both in animals and in humans.

More specifically, it has been found that the phospholipid complexes of oligomeric proanthocyanidins containing from 2 to 7 catechin units extracted from *Vitis vinifera*, prevent or reduce the formation of athero-

sclerotic plaques in a dose-depending relationship.

Such an activity was evidenced in rabbits fed with hypercholesterolemic diet, so as to induce atherosclerotic lesions similar to the human ones at the vascular level, particularly at the aortic arch, ventral aorta, carotids and cerebral vessels. In said model, the above mentioned phospholipid complexes change the macro and microscopical vascular condition reducing, compared with untreated animals, both the number of atheromatous plaques and their severity, with a surprising vascular-tissular benefit. In another atherosclerosis model, with the purpose of cerebral protection, wherein the vasal lumen of rabbit internal carotid had been surgically reduced, while administering a hypercholesterolemic diet rich in saturated fats, a decrease in carotid obstruction, a reduction of vessel walls thickness and an increased survival of the animals were evidenced. Furthermore, in atherosclerotic patients, a reduction in carotid obstruction due to atheromatous plaques and an improved carotid flow, evaluated by Doppler ultrasonography, were observed.

The phospholipid complexes of the proanthocyanidins extracted from *Vitis vinifera* can be used in suitable oral formulations, such as tablets, soft- or hard-gelatin capsules, at dosages ranging from 50 to 500 mg two-three times a day, depending on the severity of the disease to treat. The preparation of the pharmaceutical formulations can be carried out according to conventional techniques and excipients.

The following examples illustrate the invention in greater detail.

Example 1

3 groups of 8 New Zealand rabbits each, were treated as follows:

Group 1): Control, normal diet

Group 2): Hypercholesterolemic diet (0.2% cholesterol)

Group 3): Hypercholesterolemic diet + phospholipid complex of extracts of *Vitis vinifera* (0.2% cholesterol + 2% Leucoselect(R)).

After 8 weeks, during which cholesterol, LDL/VLDL, HDL and triglycerids levels were measured, the animals were killed.

The number, the size and the distribution of the atherosclerotic lesions on thoracic and abdominal aorta were evaluated.

Aorta strips were fixed and stained with Sudan IV to visualize the lesions and to evaluate the vasal cholesterol and the content in oxidized cholesterol by gaschromatography.

The results reported in the following table prove that the treatment with phospholipid complexes of extracts of *Vitis vinifera* decreases in a statistically significant way the atherosclerotic lesions induced by hypercholesterolemic diet.

Table

Treatment	area percent of the lesion
Group 1	1%
Group 2	27%
Group 3	5%*

*$p<0.01$ compared with group 2

Example 2

Capsules containing 500 mg of phospholipid complex of extracts of *Vitis vinifera*

Composition:

Complex of extract of *Vitis vinifera* with soy phosphatidylcholine	200 mg
Lactose	57 mg
Modified starch	40 mg
Magnesium stearate	3,0 mg

Example 3

Gastro-resistant tablets

Complex of extract of *Vitis vinifera* with soy phosphatidylcholine	250 mg
Microcrystalline cellulose	118 mg
Precipitated silica	3 mg
Magnesium stearate	4 mg
Methacrylic acid anionic polymer and esters thereof	12 mg
Talc	8 mg
Magnesium carbonate	8 mg
Maize star	5 mg
Gum arabic	159 mg

Example 4

Soft-gelatin capsules

Complex of extract of *Vitis vinifera* with soy phosphatidylcholine	266 mg
Peanut oil	209 mg
Partially hydrogenated vegetable oils	100 mg
Soy lecithin	5 mg

CLAIMS

1. The use of phospholipid complexes of oligomeric proanthocyanidins containing from 2 to 7 catechin units extracted from *Vitis vinifera* for the preparation of medicaments for the therapeutical and prophylactic treatment of atherosclerosis and of miocardiac and cerebral infarctions.

2. The use as claimed in claim 1, wherein the unitary dosage of the medicament ranges from 50 to 500 mg of active ingredient.

3. The use as claimed in claim 1 or 2, wherein the medicament is administrable orally.

4. The use as claimed in claim 3, wherein the medicament is in the form of soft- or hard- gelatin capsules or of tablets.

5. A method for the treatment of atherosclerosis and of miocardiac and cerebral infarctions, which comprises administering a patient with an effective amount of a phospholipid complex of extract of *Vitis vinifera*.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 98/07662

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K35/78 A61K31/35

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 963 527 A (BOMBARDELLI EZIO ET AL) 16 October 1990 cited in the application see column 2, line 38 - line 63 ---	1-5
X	EP 0 713 706 A (SUNTORY LTD) 29 May 1996 see page 3, line 28 - page 4, line 50 ---	1
A	K. TEBIB ET AL.: "ANTIOXYDANT EFFECTS OF DIETARY POLYMERIC GRAPE SEED TANNINS IN TISSUES OF RATS FED A HIGH CHOLESTEROL-VITAMIN E -DEFICIENT DIET" FOOD CHEMISTRY, vol. 59, no. 1, 1997, pages 135-141, XP002098281 --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
29 March 1999	13/04/1999

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	E. BOMBARDELLI ET AL.: "BIOLOGICAL ACTIVITY OF PROCYANIDINS FROM VITIS VINIFERA L." BIOFACTORS, vol. 6, no. 4, 1997, pages 429-431, XP002098282	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/07662

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 5
because they relate to subject matter not required to be searched by this Authority, namely:
See FURTHER INFORMATION SHEET PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claim 5 IS directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Claims Nos.: 5

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

information on patent family members

Interr nal Application No
PCT/EP 98/07662

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4963527 A	16-10-1990	AT 91688 T	15-08-1993
		CA 1337604 A	21-11-1995
		DE 3882383 A	26-08-1993
		DE 3882383 T	11-11-1993
		EP 0275224 A	20-07-1988
		ES 2056943 T	16-10-1994
		JP 1013031 A	17-01-1989
		JP 1954097 C	28-07-1995
		JP 6076335 B	28-09-1994
		PT 86509 B	31-12-1991
EP 0713706 A	29-05-1996	FI 955691 A	29-05-1996
		JP 8225453 A	03-09-1996
		NO 954834 A	29-05-1996
		US 5607965 A	04-03-1997